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EXAMINER

HUTSON, RICHARD G

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

DETAILED ACTION

Applicant's cancellation of claims 5, 13 17 and 18 and amendment of claims 1, 7, 11, 12, 14-16, 19 and 21, in the paper of 12/5/2008, is acknowledged. Claims 1-4, 6-8, 11-12, 14-16, 19 and 21 are still at issue and are present for examination.

Applicants' arguments filed on 12/5/2008, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claims 11, 12, 14-16, 19 and 21 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Claim Rejections - 35 USC § 112

The rejection of claims 1-8, 13, 18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention is hereby withdrawn based upon applicants amendment of the claims and applicants arguments presented on 12/5/2008.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

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A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 4, 6 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Liebl et al. (J. Bacteriology 174(6): 1854-1861 (1992)).

This rejection was stated in the previous office action as it applied to previous claims 1, 2, 4, 5, 6 and 8. In response to this rejection applicants have cancelled claim 5, amended claims 1 and 7 and traverse the rejection as it applies to the newly amended claims.

Applicants note that they have amended claim 1 to specify that the bacteria is selected from the group consisting of *Ralstonia eutropha*, *Pseudomonas putida* and *Escherchia coli* and applicants traverse the rejection as it applies to the newly amended claims on the following basis.

Applicants first review the legal standard, and then submit that Liebl does not recite all of the claim limitations. Specifically applicants submit Liebl does not disclose the claimed bacterial strain selected from the group consisting of *Ratstonia eutropha*, *Pseudomonas putida* and *Escherichia coli*, genetically modified to express a nuclease gene which is secreted into the periplasmic space. Applicants submit that Liebl discloses Staphylococcal nuclease (SNase) expression by various *C. glutamicum* strains, wherein the *C. glutamicum* transgenic strain is used for investigating protein export and processing.

Applicants further submit that the nuclease in Liebl is secreted into the culture medium. Applicants respectfully submit that as of the time of publication of Liebl, it was widely believed that gram positive bacteria did not have a periplasmic space (see Sakamoto, *et al.*, *Microbiology*, 147:2865-2871 (2001), submitted by Applicants with the amendment and response filed on October 24, 2007, which specifically states "gram positive bacteria have no outer membrane or periplasmic space".)

In response to this first argument presented by applicants, Liebl does disclose the claimed bacterial strain from *Escherichia coli*, specifically in Table 1, in which Liebl et al. teach the propagation of AS019/pWNuc5 in both *E. coli* and *C. glutamicum*. Thus as *E. coli* is a Gram negative strain, applicants arguments presented relative to gram positive bacteria and the presence of a periplasmic are not considered relative.

Applicants further argue that an engineered protein is not secreted into the periplasmic space of bacteria, rather one must engineer the protein by providing the necessary sequences for such secretion into the periplasmic space, as opposed to the extracellular secretion (*i.e.*, across the cell membrane and into culture medium).

In response to the argument, that an engineered protein is not secreted into the periplasmic space of bacteria, and that one must engineer the protein by providing the necessary sequences for such, applicants attention is directed to applicants claims which recite the claimed bacteria cell is genetically modified to express a heterologous nuclease gene, which Liebl et al. clearly teach as discussed above and in Table I and the supporting text. Applicants claim 1 then recites that those bacteria that express this

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heterologous nuclease must do so such that the nuclease gene product is secreted into the periplasmic space and released when the bacteria is lysed. As stated in applicant's previous response, it is clearly the case that when the bacteria are lysed, the nuclease gene product is secreted into the periplasmic space and released.

Applicant's amendment and complete traversal is acknowledged and has been carefully considered, however, is not found persuasive for the reasons previously stated and repeated herein.

Thus claims 1, 2, 4, 6 and 8 remain anticipated by Liebl et al.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-4 and 6-8, are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

This rejection was stated in the previous office action as it applied to previous claims 1-8, 13, 18. In response to this rejection applicants have cancelled claim 5, amended claims 1 and 7 and traverse the rejection as it applies to the newly amended claims.

Applicants note that they have amended claim 1 to specify that the bacteria is selected from the group consisting of *Ralstonia eutropha*, *Pseudomonas putida* and *Escherchia coli* and applicants traverse the rejection as it applies to the newly amended claims on the following basis.

Applicants first review the legal standard, and then review applicant's interpretation of what each of the references teaches. After this analysis, applicants submit that "A combination of Greer, Liebl, Miller, Atkinson and Lee does not recite all of the elements of the claims". In response to this argument applicants is reminded that this rejection is based upon the obviousness of the claims in light of the teachings of the prior art references and that it is unnecessary for the combination of Greer, Liebl, Miller, Atkinson and Lee to recite all of the elements of the claims, in order for the claims to render obvious the rejected claims.

Further, applicants is reminded that applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants further submit that Liebl and Miller disclose genetically engineering the gram positive bacteria *C. glutamicum* and *B. subtilis* respectively, to secrete nuclease into the culture medium and as noted above in response to the 102 (b) rejection, this is not tantamount to a disclosure of secretion of nuclease into the periplasmic space as claimed. In response to this argument, as is pointed out above, Liebl et al. additionally teach the genetically engineered *E. coli*.

Applicants submit that the claimed bacterial strains are engineered to (1) produce large amounts of nuclease which is (2) secreted into the periplasm where it is harmless to the cell, until release by cell lysis and that none of Greer, Atkinson or Lee makes up for these deficiencies, as Greer is not concerned with genetically engineering bacterial strains to secrete nuclease, Lee discloses the production of copolyesters in *Pseudomonas sp* and Atkinson is a review of biochemical and biotechnological methods and reagents. Applicants submit that the availability of biotechnology tools does not make obvious results obtained from their use. With respect to claim 7, none of the prior art discloses genetically modifying bacteria with the heterologous nuclease gene integrated into the chromosome, and the gene product secreted into the periplasmic space.

As above, each of applicants points are acknowledged, however, not found persuasive for the reasons of record, the reasons discussed above and for those repeated herein. Applicants are again reminded that the instant rejection is based upon obviousness as a result of the combination of the references and not anticipation and

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that thus it is not necessary that the references teach the specific points applicants argue.

One of ordinary skill in the art would have been motivated to genetically engineer a bacterial strain to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of a fermentation process for the synthesis of industrially important molecules. Applicants is reminded that Liebl et al. teach such in *C. glutamicum* and also in *E. coli*. A nuclease excreted into the medium as a result of such a genetically engineered bacterial strain would a inherently result in the degradation of at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide to be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, including *Corynebacterium glutamicum*, *Bacillus subtilis* and *E. coli*

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and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium. Alternatively one would have been motivated to engineer a homologous nuclease to increase its nuclease activity for the same reasons as stated above for the introduction of the heterologous *Staphylococcal* nuclease.

Further, one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. in order to more efficiently produce the desired product, consisting of antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides as taught by Atkinson et al. Optimization of fermentation conditions includes the choice of the bacterial host such as *Methylobacterium organophilum*, *Methylobacterium extorquens*, *Alcaligenes eutrophus*, *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Pseudomonas resinovorans*, *Pseudomonas acidovorans* and *Escherichia coli* or any other microorganism which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that *Alcaligenes eutrophus* has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32). It would have been obvious to use a bacterial strain which grows to a high cell density and/or which produces a high level of the desired product.

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Applicants point out that applicants claims are directed to a bacterial strain that are engineered to (1) produce large amounts of nuclease which is (2) secreted into the periplasm where it is harmless to the cell, until release by cell lysis, continues to be acknowledged as above, however, not considered entirely accurate. Applicants are reminded that applicants claim 1 is directed to a bacterial strain for the production of polyhydroxyalkanoates, wherein the bacterial strain is selected from a group including *E. coli* and is genetically modified to express a heterologous nuclease gene, wherein the nuclease gene product is secreted into the periplasmic space and released when the bacteria is lysed. It is noted that this claim is anticipated above by Liebl et al., as upon lysis of the bacterial strain taught by Liebl et al. the nuclease gene product is secreted into the periplasmic space and released.

Applicants argument on the basis of evidence of secondary considerations including commercial success, long felt but unresolved needs, failure of others and unexpected results, etc. is acknowledged, however, is not found persuasive for the following reasons. First it is unclear as to what if any specific "evidence of secondary considerations" applicants are referring to in their presented argument, as applicants submitted argument regarding microbial fermentations for the use of manufactured products, and needs and motivations associated with such are not considered evidence of these of secondary considerations or if they are it is unclear as to what applicants stated points evidence. Further, applicants submission of applicants discovery is not considered a secondary consideration, nor is the motivation that the skilled artisan

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would want to ultimately integrate the nuclease into the chromosome considered a secondary consideration.

Thus, these secondary considerations are further acknowledged but not found persuasive given the discussion previously and above.

Thus, claims 1-4 and 6-8, remain obvious in light of Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

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the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G. Hutson whose telephone number is 571-272-0930. The examiner can normally be reached on M-F, 7:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Nashaat T. Nashed can be reached on 571-272-0934. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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rg
3/10/2009

/Richard G Hutson/
Primary Examiner, Art Unit 1652